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入侵植物紫茎泽兰化感作用及其途径研究*

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摘要: 紫茎泽兰广泛入侵中国西南地区, 研究结果表明化感作用是其入侵的重要武器, 但其化感作用的途径并不十分清楚。本研究中, 我们发现紫茎泽兰可以通过多种途径对两种栽培植物大麦和玉米的生长产生化感作用, 这些途径包括了叶挥发物、叶淋溶物以及根分泌物。并且在紫茎泽兰幼苗早期就可以检测到这些化感作用。然而, 没有实验证据表明紫茎泽兰落叶的微生物降解物对两种测试植物具有化感作用。

关键词: 化感途径; 紫茎泽兰; 入侵植物
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Allelopathic Potential and Pathway of an Invasive Weed *Eupatorium adenophorum* (Asteraceae)

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Abstract: Crofton weed (*Eupatorium adenophorum*) is one of the most notoriously invasive plants in China. Allelopathy has been considered to play an important role for population spread. In this study, we demonstrate that this weed has strong adverse effects on two agronomic cultivars, barley and maize, through a variety of allelopathic pathways including volatiles, leachates and root exudates. The allelopathic effect was even detected in early growth stages of the weed. However, there was no evidence to indicate that allelochemicals in dead leaf tissue either persisted or converted into new allelochemicals by naturally colonizing phyllosphere microbes.

Key words: Allelopathic pathways; *Eupatorium adenophorum*; Invasive plant

Crofton weed (*Eupatorium adenophorum* Speng or *Ageratina adenophora* Sprengel) is one of the most severely invasive weeds in China. This weed first invaded the Yunnan Province of China from Burma during the 1940s, and then spread northward and eastward with an annual speed of about 20-30 km (Lu *et al.*, 2005; Wang and Wang, 2006). Currently, it commonly distributes in southwest areas of China, including Yunnan, Guizhou, Sichuan and Guangxi Provinces, and produces a great threat to the local ecological system. Besides China, more than 30 countries and areas have been invaded by *E. adenophorum* (Qiang, 1998; Lu *et al.*, 2005; Ding *et al.*, 2008).

Allelopathy is considered to be an important weapon of many invasive plants for rapidly spreading in their introduced range (Kong *et al.*, 2002; Bais *et al.*, 2003). Although there have been reports of

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the allelopathic effects of *E. adenophorumv* on different species of plants (Song *et al.*, 2000; Zheng and Feng, 2005; Li *et al.*, 2007), most of these studies tested allelopathy of *E. adenophorum* through directly extracting the chemicals from fresh below and aboveground tissues using an organic solvent (Song *et al.*, 2000; Ding *et al.*, 1999). Currently it is unclear whether allelopathic chemicals in *E. adenophorum* tissues have the ability to naturally diffuse into the environment to affect the growth of neighboring plants. Moreover, there are multiple pathways by which allelochemicals can affect other plants, including volatilization, root exudation, leaching and decomposing plant residues in soil (Ninkovic, 2003); however, little is known regarding how *E. adenophorum* affects plant growth.

In this study we tested the allelopathic effects on the growth of two agronomic cultivars, barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.). We also evaluated possible pathways involved in *E. adenophorum's* effect on plant growth, including volatiles, leaf leachates, root exudates and dead tissues of this weed.

Materials and methods

Plant Materials Fresh leaves, stems and seeds of *E. adenophorum* were collected in April from the north urban area of Kunming, Yunnan Province, China. Barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.) were chosen as indicator plants in the allelopathy bioassay, and were purchased at the market.

Allelopathy of Root Exudates, Leaf Leachates and Residues To collect *E. adenophorum* root exudates, about 200 grains of surface-sterilized seeds (>90% germination rate) were germinated on three layers of moisten filter paper in a 9 cm-diameter Petri dish at 25℃, with 12 h/12 h light/dark cycle. Filter papers were moistened by adding 10 mL of distilled water every day. Ten days after germinating, 10 mL of 1/4 MS nutrient solution was applied to support the growth of seedlings. Seedlings and root residues were removed at 2, 4 and 6-leaf stages. Filter papers were collected and dried at room temperature on a clean bench. To collect leaf leachates, fresh mature leaves obtained from the wild were soaked with distilled water (w/v: 5/100) and solutions were filtrated. To obtain allelochemical from residues, ablated and senescent leaves were collected from the wild and dried at room temperature. Crushed leaf debris was moistened with sterilized water (water content: ≈30%). Moistened leaf debris was filled into 12 flasks (50 g per bottle) and covered with plastic membranes. Leaf debris was allowed to decompose by naturally associating phyllosphere microbes at 28℃. At the following day 5, 10 and 20 the degraded residues were taken out and rapidly wind-dried at 40℃. Subsequently, allelochemicals in the residues were extracted for 10 minutes by sterilized water (w∶v=5∶100). Extracts were filtrated and condensed to 1/2 volume. All filter papers, leaf leachates and residue extracts obtained were stored at 4℃ until further use.

To test root exudate effects, two papers collected from the same layer were put into every Petri dish and evenly moistened using 10 mL of distilled water. In the other two assays, two new sterilized papers were put into each Petri dish, and a 10 mL of leaf leachate with concentrations of 50%, 100% (v∶v) or condensed residue extracts were added. Papers with 10 mL of pure distilled water were used as controls. For each assay, 30 grains of germinated barley seeds (or 20 grains for maize) were put onto filter papers in each Petri dish and incubated at 25℃ with 14 h/10 h light/dark cycle. Filter papers were moistened by supplying 10 mL of distilled water twice every day. Three repeats were prepared for each treatment.

Seedling height (SH), leaf length (LL), root length (RL) and fresh weight (FW) were measured for each seedling after 5 days' growth of indicator plant. The average values in every Petri dish were used in statistical analysis.

Allelopathy of Volatiles A glass container (diameter: 30 cm; height: 60 cm) was designed to test the allelopathy of volatiles (Fig. 1). Acting as

the donor plant, fresh materials (leaves and stems) from *E. adenophorum* were put into the lower chamber. Surface-sterilized barley seeds served as indicator plants and were cultured on moistened filter papers in Petri dishes and put in the upper chamber. Between donor and indicator plants, a sandwich structure filled with cotton or activated carbon was used to stop (filled with activated carbon) or allow (filled with cotton) *E. adenophorum* volatiles to pass through. Containers were incubated at 25℃ with 14 h/10 h light/dark cycle. Filter papers were moistened by supplying 1/4 MS nutrient solution every day. The donor plant (fresh tissues of *E. adenophorum*) was renewed every two days. After 5 and 8 days of incubation, the indicator plant was taken out to measure SH, LL and RL.

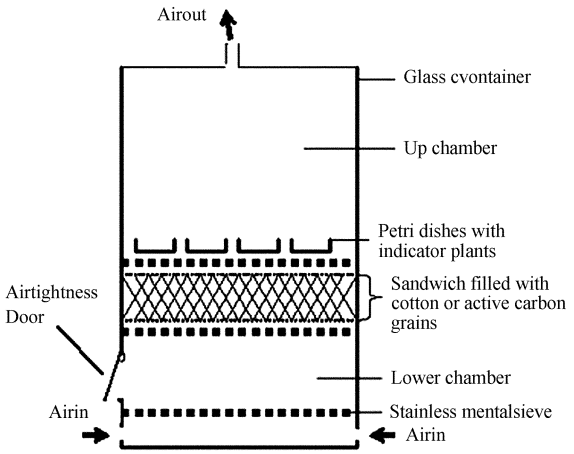

Fig. 1　The glass container used in allelopathy bioassay by volatilization pathway

Fresh tissues of donor plant (*E. adenophorum*) were put into the lower chamber and indicator plants were put into the upper chamber. The lower and upper chambers were separated by sandwich filled with cotton or activated carbon grains. Air was pumped into the chamber from the bottom and out from the top of the container. An airtightness door was designed in lower chamber for renewing donor plant tissues.

Statistical Analysis　Allelopathy potential was assessed as response index (R_I) = 1-C/T (when $T \geqslant C$) or T/C-1 (when $T<C$) according to Williamson & Richardson (1988). The parameter "C" represents the control and "T" represents the treatment. $R_I>0$ means there is an enhancing effect and $R_I<0$ means there is an inhibitory effect. The Protected Least Significant Difference (PLSD) test, using SPSS10.0 software package, was used to test statistical significance.

Results and discussion

There are many potential pathways by which *E. adenophorum* can adversely affect the growth of the indicator plant, such as root exudates, leaf leachates, and volatiles. Barley and maize were used as indicator plants to detect the allelopathic potential of *E. adenophorum* root exudates (Table 1).

Filter papers obtained from the upper to lower layers showed a decreasingly inhibitory effect on both maize and barley in seedling height, root length and plant fresh weight. Therefore, the allelpathic potential

Table 1　Response index (R_I) of *E. adenophorum* root exudates on barley and maize seedlings

Filter papers	SH		LL		RL		FW	
	barley	maize	barley	maize	barley	maize	barley	maize
Ⅰ1	−0.32**	−0.45**	−0.43**	−0.82**	−0.13	−0.35**	−0.15	−0.19*
Ⅰ2	−0.13**	−0.36*	−0.18**	−0.47**	−0.02	−0.26*	−0.06	−0.15
Ⅰ3	−0.10*	−0.16	−0.13	−0.21	−0.01	−0.06	−0.22*	−0.10
Ⅱ1	−0.15**	−0.24	−0.18**	−0.38**	−0.08	−0.09	−0.15	−0.21*
Ⅱ2	−0.10*	−0.19	−0.14*	−0.20	−0.05	−0.18	+0.10	−0.01
Ⅱ3	+0.01	−0.07	−0.01	−0.05	+0.06	−0.04	−0.02	−0.09
Ⅲ1	−0.45**	−0.12**	−0.63**	−0.16	−0.26**	−0.19**	−0.49**	−0.10
Ⅲ2	−0.13**	−0.07	−0.19**	−0.04	−0.11**	+0.09	−0.29*	−0.07
Ⅲ3	−0.01	−0.10*	+0.05	−0.11	−0.11**	−0.01	−0.27*	−0.12

* $P<0.05$; ** $P<0.01$. Ⅰ, Ⅱ and Ⅲ indicate filter papers with root exudates collected at 2, 4 and 6-leaf stages of *E. adenophorum*, respectively; 1, 2 and 3 indicate the first, second and third layer of filter paper (counting from the top layer which directly contact with the roots of *E. adenophorum*). SH: seedling height; LL: leaf length; RL: root length; FW: fresh weight

declines with decreased exudate concentrations.

Moreover, strong allelopathy of root exudates can be detected in a very early growth stage (cotyledon stage) of *E. adenophorum* seedlings, suggesting that allelopathy may play a role during its population establishment. However, we cannot determine the extent to which allelopathy helps single or few seeds establish new populations in the wild, since root exudates collected from high densities of *E. adenophorum* seedlings were tested in this experiment.

Interestedly, root exudates of cotyledon and four-leaf stages of *E. adenophorum* had nearly two times the inhibitory effect on maize than on barley seedlings. However, when root exudates of six-leaf stage were used, there was stronger inhibition in barley compared to maize seedlings. This suggests that *E. adenophorum* produces different allelochemicals during different developmental stages.

The above-ground parts of mature *E. adenophorum* also significantly inhibit the growth of indicator plants by leachates (Table 2) and volatiles (Table 3). Both long-lasting and high concentrations of leaching solution had stronger inhibition on the growth of barley seedlings. Although different growth rates were found between barley seedlings cultured in containers with cotton and those with activated carbon filled in sandwich (Table 3), volatiles had no influence on the germination ratio (data not shown). Compared to previous methods (Ninkovic, 2003), the equipment used in this study conveniently provided a way to control indicator and donor plants at an environment similar to natural atmosphere (Fig. 1).

Because *E. adenophorum* can adversely affect neighboring plants' growth by both leachates (Table 2) and volatiles (Table 3), and considering the fact that many organic chemicals previously extracted from above-ground tissues of *E. adenophorum* have been shown to be allelopathic (Song *et al.*, 2000; Li *et al.*, 1997; Ding *et al.*, 1999; Dayan *et al.*, 2003), it is possible that allelochemicals still remain active after dead leaves or stems fall back into the soil. Results indicate that allelochemicals were unlikely to persist or be converted into new allelochemicals by phyllosphere microbes, because no adverse effect was detected in fermented residues (Table 4). Interestedly, the allelopathic potential disappeared after 5 days of microbial decomposition. Based on the significant benefits to growth of barley seedlings (Table 4), the fermented residues appear to be utilized as nutrients by the indicator plant. Therefore, allelopathy via a degradation pathway unlikely occurs in *E. adenophorum* residues.

Table 2 Response index (R_I) of *E. adenophorum* leaf leachates on barley seedlings

Leaching time (s)	Concentrations (v/v) (%)	SH	LL	RL	FW
10	50	−0.137**	−0.266**	−0.040	−0.206
	100	−0.145**	−0.343**	−0.191**	−0.022
60	50	−0.145*	−0.370**	−0.315**	−0.176
	100	−0.271**	−0.568**	−0.493**	−0.382

* $P<0.05$; ** $P<0.01$. Other notes see Table 1

Table 3 Response index (R_I) of *E. adenophorum* volatiles on barley seedlings

Treating duration	SH	LL	RL
5 days	−0.035	−0.053	−0.072
8 days	−0.055	−0.121**	−0.097*

* $P<0.05$; ** $P<0.01$. Other notes see Table 1

To date, strategies including applying pathogen (Dai *et al.*, 2004) or gall (Li *et al.*, 2006) controls, and native plant replacement (Wang *et al.*, 2006) have been used to block the spread of *E. adenophorum* in southwest China, but none has been proven to be effective. The finding that allelochemicals in *E. adenophorum* can be completely degraded by microbes offers a potentially effective way to control this weed: harvest it as organic fertilizer after microbial fermentation.

In this study, allelopathy for two agricultivars existed under experimental conditions, however, it is yet to be determined that allelopathy facilitates *E. adenophorum* spread in the wild. In the future, growth inhibition to several native plants should be evaluated.

Table 4　Response index (R_I) of *E. adenophorum* leaf residues on barley seedlings

Degrading days	SH		LL		RL		FW	
	5 mL	10 mL	5 mL	10 mL	5 mL	10 mL	5 mL	10 mL
0	−0.25 **	−0.44 **	−0.39 **	−0.46 **	−0.47 **	−0.79 **	−0.37 **	−0.91 **
5	+0.188 **	+0.285 **	+0.235 **	+0.335 **	+0.191 **	+0.198 **	+0.188	+0.167
10	+0.202 **	+0.239 **	+0.258 **	+0.284 **	+0.129 *	+0.068	+0.185	+0.068
20	+0.226 **	+0.280 **	+0.290 **	+0.326 **	+0.132 **	+0.130 **	+0.031	+0.144

* $P<0.05$; ** $P<0.01$.　5 mL and 10 mL indicate the volume of condensed solution used in bioassay. Other notes see Table 1.

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